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1 **Efficacy of fecal sampling as a gut proxy in the study of chicken gut microbiota**

2

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11 **Abstract**

12 **Background:** Despite the convenience and noninvasiveness of fecal sampling, the fecal
13 microbiota does not fully represent that of the gastrointestinal (GI) tract, and the efficacy of fecal
14 sampling to accurately represent the gut microbiota in birds is poorly understood. In this study, we
15 aim to identify the efficacy of feces as a gut proxy in birds using chickens as a model. We
16 collected 1,026 samples from 206 chickens, including duodenum, jejunum, ileum, cecum and
17 feces samples, for 16S rRNA amplicon sequencing analyses.

18 **Results:** In this study, the efficacy of feces as a gut proxy was partitioned to microbial community
19 membership and community structure. Most taxa in the small intestine (84.11 – 87.28%) and ceca
20 (99.39%) could be identified in feces. Microbial community membership was reflected with a gut
21 anatomic feature, but community structure was not. Excluding shared microbes, the small intestine
22 and ceca contributed 34.12 and 5.83% of the total fecal members, respectively. The composition
23 of Firmicutes members in the small intestine and that of Actinobacteria, Bacteroidetes, Firmicutes
24 and Proteobacteria members in the ceca could be well mirrored by the observations in fecal
25 samples (ρ = 0.54 – 0.71 and 0.71 – 0.78, respectively, $P < 0.001$). However, there were few
26 significant correlations for each genus between feces and each of the 4 gut segments, and these
27 correlations were not high (ρ = -0.2 – 0.4, $P < 0.05$) for most genera.

28 **Conclusions:** Our results provide evidence that the good potential of feces to identify most taxa in
29 chicken guts, but it should be interpreted with caution by using feces as a proxy for gut in
30 microbial structure analyses. This work provides insights and future directions regarding the usage
31 of fecal samples in studies of the gut microbiome.

32 **Keywords:** gut microbiota, feces, proxy, spatial relationships, chicken

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33 **Background**

34 Many studies have reported the important roles of gut microbiota in host metabolism and health in
35 humans [1], other mammals [2] and birds [3]. Because of the convenience and noninvasiveness of
36 fecal sampling, most studies use fecal samples as a proxy to study the gut microbiota, despite the
37 increasing recognition that fecal microbial populations may not be fully representative of those in
38 the contents or mucosa of the gastrointestinal (GI) tract [4, 5]. Therefore, a comprehensive
39 understanding of the efficacy of using fecal samples as a proxy to study the GI microbiota would
40 help improve longitudinal analyses of microbiota and the application of fecal samples [6, 7].

41 Among birds, the chicken is frequently used as a research model, and its GI microbiota has been
42 studied previously [8-12]. In several studies, the microbiota present in different GI segments have
43 been investigated using traditional sequencing methods [13] or high-throughput sequencing
44 techniques [14, 15]. However, these studies had small sample sizes (N = 3 – 8), were primarily
45 aimed at examining the spatial heterogeneity among different segments and did not focus on the
46 spatial microbiota relationships between feces and the GI tract.

47 Compared with most mammals, the cecum in birds has been reported to play important roles in
48 metabolism, such as in the digestion of cellulose, starch and other resistant polysaccharides [16,
49 17] and in the absorption of nutrients [18] and water [19]. Microbial compositions and functions in
50 chicken ceca have been reported in many studies [20, 21]. In addition, Stanley et al. [22] examined
51 the microbial relationships between the ceca and feces and observed that 88.55% of all operational
52 taxonomic units (OTUs) were shared. However, the microbial relationships between the ceca and
53 small intestine (including the duodenum, jejunum and ileum), which would help provide an
54 integrated view of gut microbial relationships, were rarely reported.

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55 Here, we performed large-scale sequencing surveys and focused on the efficacy of using feces
56 to represent the GI microbiota in chickens. The efficacy was partitioned into microbial community
57 membership and structure to gain a comprehensive view to improve our understanding of the
58 efficacy of the use of feces as a proxy to study the gut microbiota and their spatial relationships in
59 the gut.

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60 **Results**

61 **Sequencing data**

62 The 16S rRNA gene-based sequencing from 206 chickens produced 62,193,309 reads, 58,959,487
63 of which remained after quality filtration. The average number of sequences per sample was
64 57,465 and the number of sequences per sample ranged from 22,321 to 224,188.

65

66 **Landscape and quantification of microbial relationships among feces, ceca and small**
67 **intestine**

68 To gain an overview of the microbial relationships among the chicken duodenum, jejunum, ileum,
69 ceca and feces, unweighted UniFrac distances (community membership; presence/absence of taxa)
70 and weighted UniFrac distances (community structure; taking the relative abundances of taxa into
71 account) were used to perform principal coordinates analysis (PCoA; Fig. 1A, B). The variation in
72 community memberships among different sites were primarily explained by the sites origin (Fig.
73 1A), but the community structures showed both the sites origin and interindividual variation (Fig.
74 1B). In particular, the cecal microbial community exhibited a distant relationship with the small
75 intestine community, and the microbial community in feces showed an intermediate relationship
76 between those of the ceca and small intestines.

77 UniFrac distances between two samples from all assayed sites within each individual were
78 calculated to quantify the spatial relationships of the gut microbiota. When the community
79 membership was considered alone, the UniFrac distance decreased along the gut anatomical
80 locations from the farthest to the nearest sites between fecal and duodenal, jejunal, ileal or cecal
81 samples (FD, FJ, FI or FC, respectively, in Fig. 1C), presenting clear anatomical differences.

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82 However, when taking the community structure into account, the UniFrac distance increased in FI
83 and FC compared with that in FJ (Fig. 1D). This finding might be explained by the exchange of
84 contents between the ileum and ceca, suggesting that the specific cecal microbial structure
85 influences the microbial communities in the ileum and feces.
86 Among all pairs, the unweighted UniFrac distance between the cecal and duodenal as well as
87 jejunum samples were highest ($P < 0.05$), and that between duodenal and jejunal samples was
88 lowest ($P < 0.05$; Fig. 1C and Additional file 1-2: Table S1-S2). Regarding the weighted UniFrac
89 distances, cecal samples had similar distances to the duodenal and jejunal samples, and these
90 distances were greater than for the other pairs ($P < 0.05$), whereas the lowest distance was
91 observed between duodenal and jejunal samples ($P < 0.1$; Fig. 1D and Additional file 1-2: Table
92 S1-S2). These results suggest that limited differences exist within small intestinal microbial
93 communities, while the microbial structure in the ceca is quite distinct from those in the small
94 intestine.
95
96 **Analyses of shared and exclusive microbial members**
97 Given that both community membership and structure influence the microbial relationships among
98 the feces, ceca and small intestine, we next evaluated the extent to which the spatial relationships
99 were influenced by the above two factors. The shared and exclusive OTUs were calculated to
100 assess the influence of the microbial community membership. To decrease the data noise, only
101 OTUs present in more than 3 samples at each sampling site were used to analyze the effect of
102 microbial membership. We observed that 971 OTUs, accounting for 30.9% of the total OTUs,
103 were shared across all sites (Fig. 2A), and these shared OTUs can be referred to as the "core"

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104 microbiota in the gut. These OTUs represented different proportions of sequences in different sites
105 and were especially high in fecal samples (96.50%; Fig. 2B), indicating that the most abundant
106 members detected in fecal samples belonged to these "core" microbiota. At the genus level, these
107 core taxa were primarily classified as *Bacteroides*, *Intestinibacter*, *Lactobacillus*, *Rikenellaceae*
108 *RC9 gut group* and *Gallibacterium* (Additional file 3: Figure S1A). It is noteworthy that 5.88% of
109 the "core" microbiota sequences were not assigned and that most of these sequences (71.40%)
110 were detected in the cecal samples (small pie chart in Additional file 3: Figure S1A), suggesting
111 that most of these unassigned taxa tended to be anaerobic microbes.
112 Most OTUs in the small intestine (84.11 – 87.28%) and cecal (99.39%) samples could be
113 identified as fecal OTUs (Table 1), indicating that feces would be a good proxy for identifying
114 species in the gut microbiota. However, some OTUs that were present in the GI tract (12.72 –
115 15.89% in small intestinal and 0.61% in cecal samples) remained undetected in fecal samples
116 (Table 1) and members of *Clostridiales*, *Rhizobiales*, *Xanthomonadales* and *Bacteroidales*
117 appeared to be particularly undetected in feces (Additional file 4: Table S3).
118 Microbial communities in the small intestine and ceca did not contribute equally to the fecal
119 microbial members, as 35.18% of fecal OTUs were not identified in cecal samples, most of which
120 (34.12%) could be identified in small intestinal niches (Table 1, Fig. 2C). These OTUs were
121 primarily from the orders *Clostridiales*, *Lactobacillales*, *Pseudomonadales*, *Rickettsiales* and so
122 on (Additional file 3: Figure S1B) and were considered exclusive contributors of the small
123 intestinal microbiota to fecal microbial members. The ceca exclusively contributed 5.83% of
124 OTUs to the observed fecal members, representing 0.28% of the fecal sample sequences and
125 consisting of taxa primarily from the orders *Bacteroidales*, *Rhizobiales*, *Clostridiales*,

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126 *Micrococcales* and *Flavobacteriales* (Fig 2C and Additional file 3: Figure S1C).

127

128 **Correlation analyses of microbial abundances**

129 Because community structure also affects the spatial relationships of gut microbiota, we next
130 performed Spearman correlation analyses between the mean fecal and segmental genera
131 abundance to evaluate the effects of community structure and assess the extent to which the
132 microbial community in the GI tract was reflected in the fecal samples (Fig. 3). If a high
133 correlation was observed between two sites, the differences in abundance between sites were
134 considered highly consistent, so that the abundance at one site had the potential to be a good proxy
135 for the abundance at another. The microbial composition of feces was correlated with those in the
136 small intestine (Spearman: $\rho = 0.38$; $P < 0.001$) and in the combination of small intestine and ceca
137 ($\rho = 0.48$; $P < 0.001$; Fig. 3). We then performed similar analyses to identify the correlation bias in
138 predominant phyla (Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria; Additional file 5:
139 Figure S2). Genera of the Firmicutes and Proteobacteria phyla in fecal samples showed moderate
140 to high correlations with those at all four GI sites ($\rho = 0.40 - 0.76$, $P < 0.001$). In particular, fecal
141 samples were well representative of Firmicutes members in both the small intestine and ceca ($\rho =$
142 $0.54 - 0.71$, $P < 0.001$) and of Actinobacteria, Bacteroidetes and Proteobacteria members in the
143 ceca ($\rho = 0.74 - 0.78$, $P < 0.001$). However, Actinobacteria members in the small intestine might
144 not be well represented in fecal samples ($\rho = 0.13 - 0.22$, $P > 0.05$).

145 A follow-up question concerned the extent to which each microbe correlated between two sites.
146 To address this issue, Spearman correlation tests were performed for each genus between two sites.
147 The genera with abundances over 0.1% at either compared site with a significant correlation ($P <$

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148 0.05) are summarized in Fig. 4 and Additional file 6: Table S4. Between the fecal and each of the 4
149 gut segmental samples, a limited number of significant correlations ($P < 0.05$) were observed, and
150 these correlations were not high (ρ = -0.2 – 0.4, $P < 0.05$) for each genus. Most genera with
151 significant correlations belonged to the phyla Firmicutes and Proteobacteria. However, more
152 significant and moderate correlations were observed between two of the small intestinal segments,
153 and most of the genera with significant correlations were also from the phyla Firmicutes and
154 Proteobacteria (Additional file 6: Table S4). The results suggest that the gut microbiota structures
155 could be moderately reflected by fecal samples when taking all genera into consideration
156 simultaneously, but analyses of fluctuations in abundance for a specific genus should be
157 interpreted with caution.

158 Although microbes at one site were weakly correlated with the corresponding microbes at
159 another site, certain patterns were observed in some cases, as exemplified by the genus
160 *Campylobacter* (Additional file 6: Table S4). The abundance of this genus in ceca exhibited
161 consistent correlations with that observed in the jejunum ($\rho = 0.21$, $P < 0.05$) and ileum ($\rho = 0.37$,
162 $P < 0.05$). In ileal samples, this genus was correlated with that measured in fecal samples (ρ =
163 0.19, $P < 0.05$), while no correlation was observed between cecal and fecal samples. This finding
164 indicates that *Campylobacter* has great colonization ability in the distal gut of chickens, especially
165 in ceca, and most *Campylobacter* contributions to the fecal composition are probably from the
166 ileum, but not from the ceca.

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167 **Discussion**

168 This study is a large-scale sequencing assessment of the efficacy of using fecal samples as a proxy
169 for the gut microbiota in birds. In this study, we comprehensively examined the community
170 membership and structure of the chicken gut microbiome at five different biogeographic sites
171 within 206 individual animals. We showed that fecal samples were good proxies for detecting the
172 presence/absence of GI microbial members because most GI tract members could be detected
173 within anatomic features in fecal samples (microbial communities in feces showed increasing
174 similarities to those in the GI tract along the duodenum-jejunum-ileum-ceca axis). However, phyla
175 bias and interindividual effects were observed to affect the efficacy of using fecal samples to study
176 GI microbial abundance.

177 We also should note that the next-generation sequencing (NGS) approach could not absolutely
178 detect all microbes in the gut because of some limitations of NGS method [23, 24]. Some
179 microbes that may be present at lower levels than the limit of detection. Therefore, some OTUs
180 that were not detected in feces but were found in the small intestine or ceca probably exist but
181 remain below the detection limit or filtration criteria.

182 Similar to the current study, a high proportion of shared OTUs has been previously observed
183 between fecal and cecal samples in chickens [22]. Similarly, a study in house mice observed that
184 93.3% of OTUs were shared between fecal and lower GI samples [25]. Another chicken study
185 indicated that the GI origin is a primary determinant for the chicken fecal microbiota composition
186 [26], supporting the high proportion of shared OTUs between feces and the four gut segments
187 observed in the current study. These results indicate that fecal samples have good potential for
188 identifying microbial members derived from the GI tract. However, another chicken study by Choi

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189 et al. [14] observed low percentages of shared OTUs between segments. A major reason for the
190 differences among studies might be the small sample size in Choi's study, which would increase
191 the sensitivity of the results with respect to individual variation. Moreover, the presence/absence
192 of microbial members in the GI tract was observed to be reflected by fecal samples in a given
193 anatomical feature, i.e., fecal samples had more similarities in community membership to those in
194 ileal and cecal samples than to those in duodenal and jejunal samples, consistent with previous
195 reports in birds [15] and mammals [25, 27, 28].

196 As for microbial community structure, the efficacy of using fecal samples to represent the gut
197 microbiota structure did not work as well as for community membership. First, the weighted
198 UniFrac distances between feces and each of intestinal segments were significantly higher than the
199 corresponding unweighted UniFrac distances (Additional file 7: Figure S3), suggesting that taking
200 the abundance into account significantly increased the dissimilarity between feces and each of the
201 GI segments. Second, the abundances of most taxa were significantly different between fecal and
202 GI samples (Additional file 8: Table S5), consistent with previous studies[13, 15, 29]. Third, the
203 correlations between the mean fecal and segmental genera abundances were moderate, similar to
204 the results in rhesus macaques [30]. However, these correlations display bias among different
205 phyla, i.e., different phyla in the GI tract are differentially mirrored by fecal samples. Fourth,
206 significant correlations ($P < 0.05$) of each microbe between fecal and segmental samples were low
207 and rare, suggesting that the efficacy of using fecal samples to represent microbial abundance was
208 affected by the interindividual effect. A similar effect has also been observed in humans [4].

209 Previous studies in humans [4, 31] and other mammals [30, 32] have also addressed the issue of
210 whether fecal samples are good representatives for GI microbial analyses. Although the

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211 conclusions may not be fully consistent, nearly all studies reached a consensus that microbial
212 communities in fecal samples do not represent the whole GI microbiota. Studies in humans
213 suggest that microbial communities in the duodenum and colon are not represented by those in
214 feces because of the large differences in microbial profiles [31], and these studies emphasized the
215 need to examine tissue biopsies in addition to fecal samples [5], proposing that standard forceps
216 mucosal biopsy samples can represent bacterial populations [4]. Compared with human studies,
217 studies in other mammals are more comprehensive because a larger number of gut segments can
218 be involved in the analyses. Several studies in mice [25, 32] support the utility of fecal samples for
219 studying the gut microbiota, because microbial communities in fecal samples were observed to be
220 similar to those in the lower GI tract, which is supported by studies conducted in rhesus macaques
221 [30], pigs [33] and equines [34].

222 Compared with previous studies, the strength of the current study lies in the following: 1) it
223 involved the use of gut segments from the upper GI tract to the lower GI tract and feces, providing
224 a relatively comprehensive view of the spatial relationships of the gut microbiota; 2) the microbial
225 relationships were partitioned into two parts, i.e., microbial community membership and structure,
226 providing multiangle observations to identify microbial relationships between feces and the GI
227 tract; and 3) a massive number of individuals was sampled, which is significant for investigations
228 of gut spatial relationships, as the sizes of most of the above studies did not exceed twenty. The
229 considerable sample size would provide more comprehensive insights into exploring the utility of
230 fecal samples in studies of the gut microbiota.

231 Because of the specific and significant roles in nutrition and health [17, 35], ceca have been
232 widely investigated in birds [36, 37], especially chickens [20, 21, 38]. *Bacteroides* was observed

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233 as the dominant taxa in our study (Additional file 3: Figure S1D) and in most other studies [39,
234 40], although some reports observed a predominance of *Clostridiales* members in ceca [14, 41].
235 Although the cecal microbial community may sometimes be linked to diet [36], the nearly
236 consistent results across studies suggests that the cecal microbial community is stable. This
237 finding might be due to ceca having a special blind-ended structure and being located in the lower
238 GI tract, providing a stable and anaerobic environment for microbes and longer storage periods of
239 the contents, in contrast to the rapid transit environment in the small intestine [42]. In addition to
240 the microbial composition, Stanley et al. [22] also compared microbial differences and similarities
241 between ceca and feces in chicken. They observed that 88.55% of all OTUs, containing 99.25% of
242 all sequences, were shared by the ceca and feces, similar to the observations in the current study.
243 These results indicate that except for some rare microbial members, most microbes in the ceca can
244 be detected in fecal samples.

245 The microbial relationships between the ceca and small intestine have been rarely reported in
246 birds. Choi et al. [14] compared the percentage of shared OTUs among ceca and three small
247 intestinal sections but observed low percentages between segments (ranging from 1.2 to 2.9%,
248 representing from 38.7 to 65.5% of sequences). The percentages reported in another study (60.2%
249 for the duodenum, 50.5% for the jejunum and 43.5% for the ileum, which were calculated from
250 Figure 3 in their article) were higher than those in Choi's study. In contrast, the results of Xiao's
251 study presented an opposite trend from our findings, i.e., the percentages of shared OTUs in
252 Xiao's study decreased from the duodenum to the jejunum and ileum, demonstrating a
253 reversed-anatomical feature compared with the current study. These inconsistent results might be
254 attributable to differences among species, diets or other environmental factors, but the small

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255 sample size in Xiao's study may be an important reason for these inconsistencies.

256

257 **Conclusion**

258 Overall, we assessed the efficacy of using fecal samples to represent GI microbiota in birds and
259 analyzed potential factors affecting this efficacy. With highly shared microbial members, fecal
260 samples have the good potential to be used to detect most microbial species in the small intestine
261 and ceca with gut anatomical features. However, analyses of microbial structures using fecal
262 samples as the proxy for the gut in longitudinal microbial studies should be interpreted with
263 caution. This study attempts to identify the microbial relationships between feces and the intestine
264 in birds, which will help extend our understanding of the bird gut microbiota and provide future
265 directions regarding the usage of fecal samples in studies of the gut microbiome.

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266 # Methods

267 ## Animal model

268 The complete procedure was performed according to the guidelines established by the Animal
269 Care and Use Committee of China Agricultural University (permit number: SYXK 2013-0013).

270 The slow-growing yellow broiler was used as the animal model in this study, and the birds were
271 obtained from Wen's Nanfang Poultry Breeding Co., Ltd. in Guangdong Province of China. Two
272 hundred and six birds with similar body weights were selected and raised on the ground with *ad*
273 *libitum* feeding and nipple drinkers. The birds were fed a common maize-soybean-based diet
274 throughout the duration of the experiment. No antibiotics were applied during the thirty-five days
275 before sample collection. Because chickens are the largest population of birds on earth, the
276 chicken was selected as a bird model for this investigation. The slow-growing yellow broiler has
277 not been highly selected for production, making this breed of chicken closer to the ancestral birds.

278

279 ## Sample collection

280 Fresh fecal samples were collected from each bird as soon as excreta was discharged through the
281 cloaca at 77 days of age with the average body weight was 2.32 kg. Next, all the birds were
282 humanely euthanized by cervical dislocation and subsequently dissected. The contents and
283 mucosal surfaces of the duodenum, jejunum, ileum and cecum were collected immediately after
284 dissection. To ensure the consistency of samples among individuals, a 10-cm-long fixed section of
285 the duodenum and jejunum, the whole ileum and a pair of ceca were selected for sampling from
286 each bird. The contents and mucosa were mixed uniformly before collection. All samples were
287 immediately placed in liquid nitrogen and then stored at -80°C. Both the intestinal contents and

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288 mucosa were sampled based on the consideration that the microbes from both sources may
289 contribute to host interactions with respect to nutrient metabolism and immunity [43].
290

291 **DNA extraction and 16S rRNA gene sequencing**

292 DNA was extracted from intestinal and fecal samples using a QIAamp DNA stool mini kit
293 (QIAGEN, cat#51504) [44] following the manufacturer's instructions. PCR amplification of the
294 V4 region of the bacterial 16S rRNA gene was performed using the forward primer 520F
295 (5'-AYTGGGYDTAAAGNG-3') and the reverse primer 802R
296 (5'-TACNVGGGTATCTAATCC-3'). Sample-specific 7-bp barcodes were incorporated into the
297 primers for multiplex sequencing. The PCR reactions contained 5 μl of Q5 reaction buffer (5×), 5
298 μl of Q5 High-Fidelity GC buffer (5×), 0.25 μl of Q5 High-Fidelity DNA Polymerase (5 U/μl), 2
299 μl of dNTPs (2.5 mM), 1 μl (10 μM) of each forward and reverse primer, 2 μl of DNA template,
300 and 8.75 μl of ddH_2O. Thermal cycling consisted of initial denaturation at 98°C for 2 min,
301 followed by 25 cycles of denaturation at 98°C for 15 s, annealing at 55°C for 30 s, and extension
302 at 72°C for 30 s, with a final extension at 72°C for 5 min. PCR amplicons were purified using
303 Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified using a PicoGreen
304 dsDNA Assay kit (Invitrogen, Carlsbad, CA, USA). After the quantification step, amplicons were
305 pooled in equal amounts, and 2 × 300 bp paired-end sequencing was performed using an Illumina
306 MiSeq platform with the MiSeq Reagent kit v3 at Shanghai Personal Biotechnology Co., Ltd.
307 (Shanghai, China). The raw data on which the conclusions of the manuscript rely have been
308 deposited in the National Center for Biotechnology Information (NCBI) database (accession
309 numbers SRP139192, SRP139193 and SRP139195).

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Analysis of sequencing data

Data analysis was performed using the Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) pipeline [45], due to the advantages of QIIME [46-48]. Briefly, raw sequencing reads with exact matches to the barcodes were assigned to respective samples and identified as valid sequences. The low-quality sequences were filtered based on the following criteria [49, 50]: length < 150 bp, average Phred score < 20, ambiguous bases, and mononucleotide repeats > 8 bp. Paired-end reads were assembled using FLASH [51], and chimera detection was performed with QIIME. After quality control, four fecal samples were excluded due to low sequence quality that was potentially caused by a technical artifact. The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence identity using an open-reference OTU picking protocol against the Silva database (SILVA128) [52-54].

We focused on open-reference OTU picking for these analyses because this method yields substantially more taxonomic identifications with sequences that failed to hit the reference database than do closed-reference methods [55]. The open-reference method can provide more information for comparisons among intestinal segments or feces. The singleton OTUs were discarded because such OTUs can occur due to sequencing errors. Only OTUs representing more than 0.001% of the total filtered OTUs were retained to improve the efficiency of the analysis. Because the sequencing and sampling quantity varied among individuals, we rarefied the data to the lowest numbers of sequences per sample to control for sampling effort in diversity analyses. Alpha and beta diversity of individual OTUs were calculated with postrarefaction data and the phylogenetic tree. Principal coordinate analysis (PCoA) was performed using the unweighted or

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332 weighted UniFrac distance [56] for different intestinal segments and feces. To decrease the data
333 noise, only OTUs that were present in more than 3 samples at each sampling site were used to
334 analyze the effect of microbial membership. The correlations between the mean fecal and
335 segmental genera abundance were calculated using the method described in a study of rhesus
336 macaques [30]. These methods could primarily provide the number and diversity of microbes in
337 feces and each segment which would help to quantitatively understand the relationships of
338 microbial communities between feces and GI tract.
339
340 **Statistical analysis**
341 Venn plots were generated for intestinal segment or feces samples at the OTU level using the
342 VennDiagram package in **R**. Spearman correlation analysis was performed in package psych in **R**.
343 Paired Student's *t*-test was used to compare the microbial UniFrac distance between two sampling
344 sites. Mann-Whitney test was performed to identify the differences of each genus between two
345 sampling sites.
346
347
348 **Abbreviations**
349 GI: Gastrointestinal; FD, FJ, FI and FC: The unweighted or weighted UniFrac distance between
350 feces and duodenum, jejunum, ileum or cecum, respectively; NGS: Next-generation sequencing;
351 OTU: Operational taxonomic unit.

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352 ## Declarations

353 ***Ethics approval and consent to participate***

354 The complete procedure was performed according to the guidelines established by the Animal

355 Care and Use Committee of China Agricultural University (permit number: SYXK 2013-0013).

356

357 ***Consent for publication***

358 Not applicable

359

360 ***Availability of data and material***

361 The raw data on which the conclusions of the manuscript rely has been deposited in the National

362 Center for Biotechnology Information (NCBI) database (accession number SRP139192,

363 SRP139193 and SRP139195).

364

365 ***Competing interests***

366 The authors declare no conflicts of interest.

367

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370 Research in University (IRT_15R62) and China Agriculture Research System (CARS-40).

371

372 ***Authors' contributions***

373 WY, JXZ and NY designed the study. WY, JXZ, CLW, CLJ, DXZ, YHC and CJS collected the

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374 samples. WY analyzed the data and wrote the manuscript. CLW assisted in construction of the
375 figures. CJS and NY assisted in data analyzing and contributed to the revisions. All authors read
376 and approved the final manuscript.
377
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526 **Figure Legends**

527 **Fig. 1** Site origin and inter-individual effects on the shape of microbial community
528 membership and structure. (A) Principal coordinates analysis (PCoA) with
529 unweighted UniFrac distance. Each dot represents a sample from duodenum (D),
530 jejunum (J), ileum (I), cecum (C) or feces (F). PC1 and PC2 represent the top two
531 principal coordinates that captured the most variation, with the fraction of variation
532 captured by that coordinate shown as a percent. (B) PCoA plot with weighted UniFrac
533 distance, similar to (A). (C) Unweighted UniFrac distance (mean ± SEM) between
534 two sampling sites. DJ represents the UniFrac distance between the duodenal and
535 jejunal microbial community, and it was the same as DI, JI, CD, CJ, CI, FD, FJ, FI
536 and FC. Asterisks indicate the significance of the paired *t*-test: ***$P < 0.001$, **$P <$
537 0.01, *$P < 0.05$ and ˙$P < 0.1$. (D) Weighted UniFrac distance between two sampling
538 sites, similar to (C).

539

540 **Fig. 2** OTUs shared across different sampling sites. (A) Venn diagram demonstrating
541 that the taxa overlap among different sampling sites. (B) The percentage of core
542 OTUs and sequences represented by these OTUs in the duodenal (D), jejunal (J), ileal
543 (I), cecal (C) and fecal (F) samples. (C) The percentage of OTUs in feces exclusively
544 contributed by small intestine or cecum, and the percentage of OTUs in feces was
545 below the limit of detection in the gastrointestinal tract.

546

547 **Fig. 3** Microbial compositions in feces mirror those in the gastrointestinal tract. Each

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548 dot represents a genus. The average relative abundance of each genus in feces is
549 transferred by negative logarithm and shown at x-axis. The average relative abundance of
550 each genus in small intestine (SI) or intestine including small intestine and ceca (SI + C) is
551 transferred by negative logarithm and shown at y-axis. Spearman's rho was calculated with the
552 negative logarithm-transferred relative abundances between feces and SI (or SI + C).
553
554 **Fig. 4** Distribution of Spearman correlations for each genus between two sites. D, J, I,
555 C and F denote the microbial communities of the duodenum, jejunum, ileum, cecum
556 and feces, respectively. Only genera with an abundance > 0.1% at either site of
557 comparison and significant correlations ($P < 0.05$) are shown.

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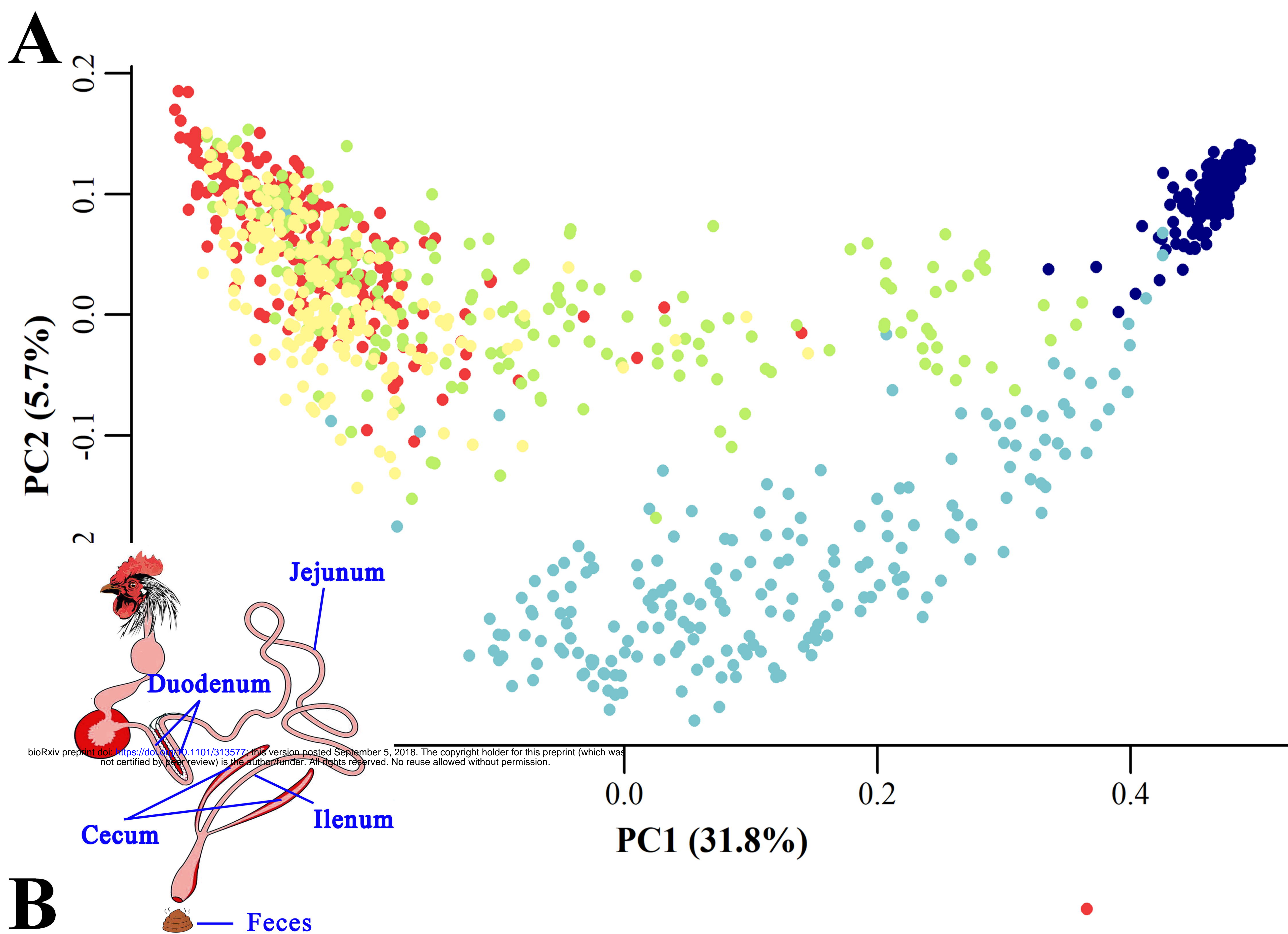
558 **Table 1** Shared and exclusive OTUs between two of sampling sites

Site1	Site2	Shared OTUs		Exclusive OTUs	
		In site1, %	In site2, %	In site1, %	In site2, %
D[1]	J	94.73[2]	93.83	5.27	6.17
		99.86[3]	99.94	0.14	0.06
D	I	96.99	80.84	3.01	19.16
		99.57	99.28	0.43	0.72
J	I	97.35	81.92	2.65	18.08
		99.79	99.48	0.21	0.52
C	D	61.14	45.78	38.86	54.22
		92.78	35.19	7.22	64.81
C	J	62.53	46.38	37.47	53.62
		93.28	41.86	6.72	58.14
C	I	89.34	55.75	10.66	44.25
		99.17	54.75	0.83	45.25
F	D	73.27	84.11	26.73	15.89
		98.57	96.94	1.43	3.06
F	J	74.36	84.55	25.64	15.45
		98.69	97.84	1.31	2.16
F	I	91.22	87.28	8.78	12.72
		99.23	97.02	0.77	2.98
F	C	64.82	99.39	35.18	0.61
		98.13	99.96	1.87	0.04

559 [1]D, J, I, C and F denotes the microbial community of duodenum, jejunum, ileum, cecum and feces,
560 respectively. [2]The percentage of shared or exclusive OTUs; [3]The percentage of sequences shared
561 or exclusive OTUs represent.



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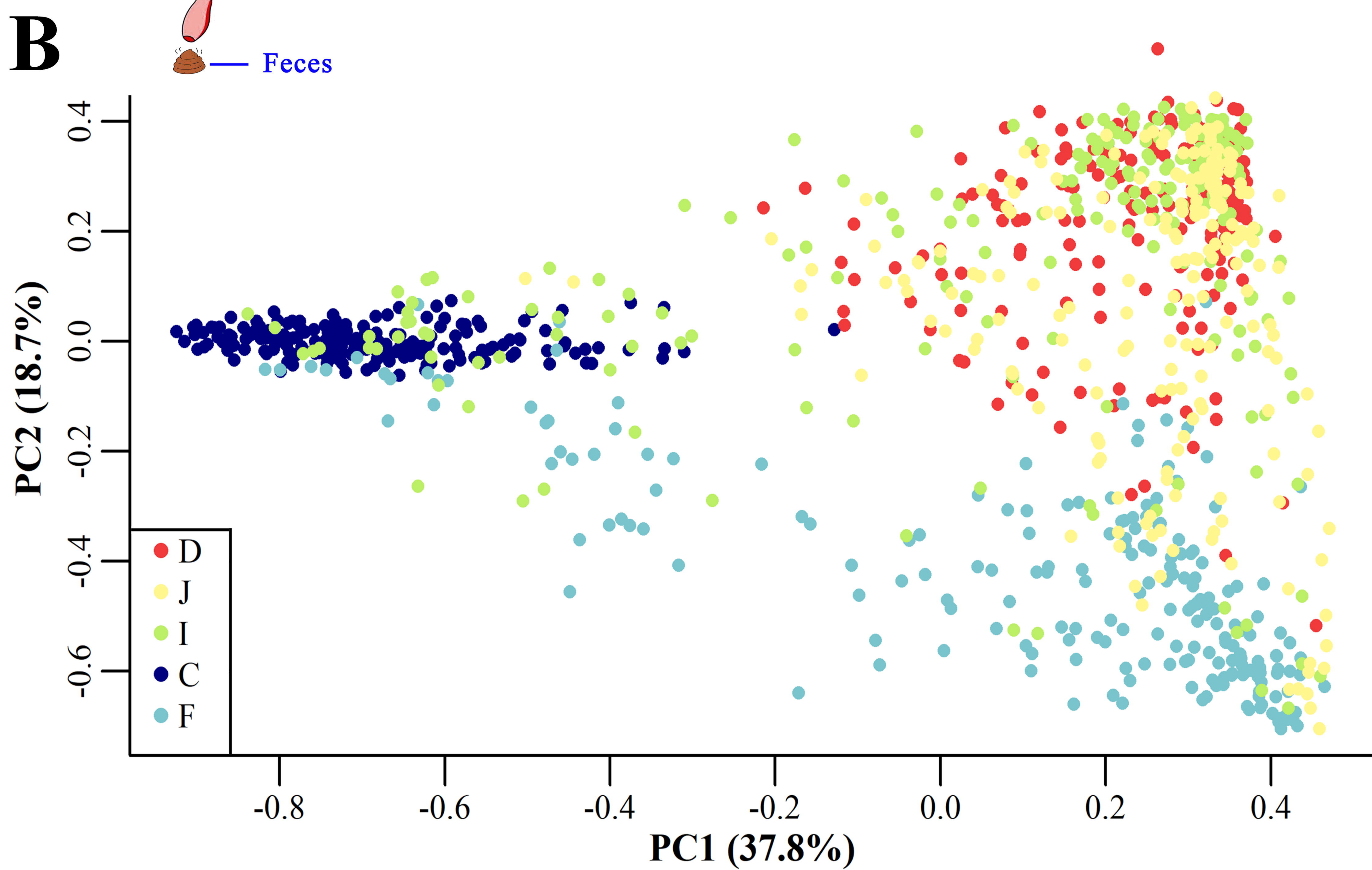

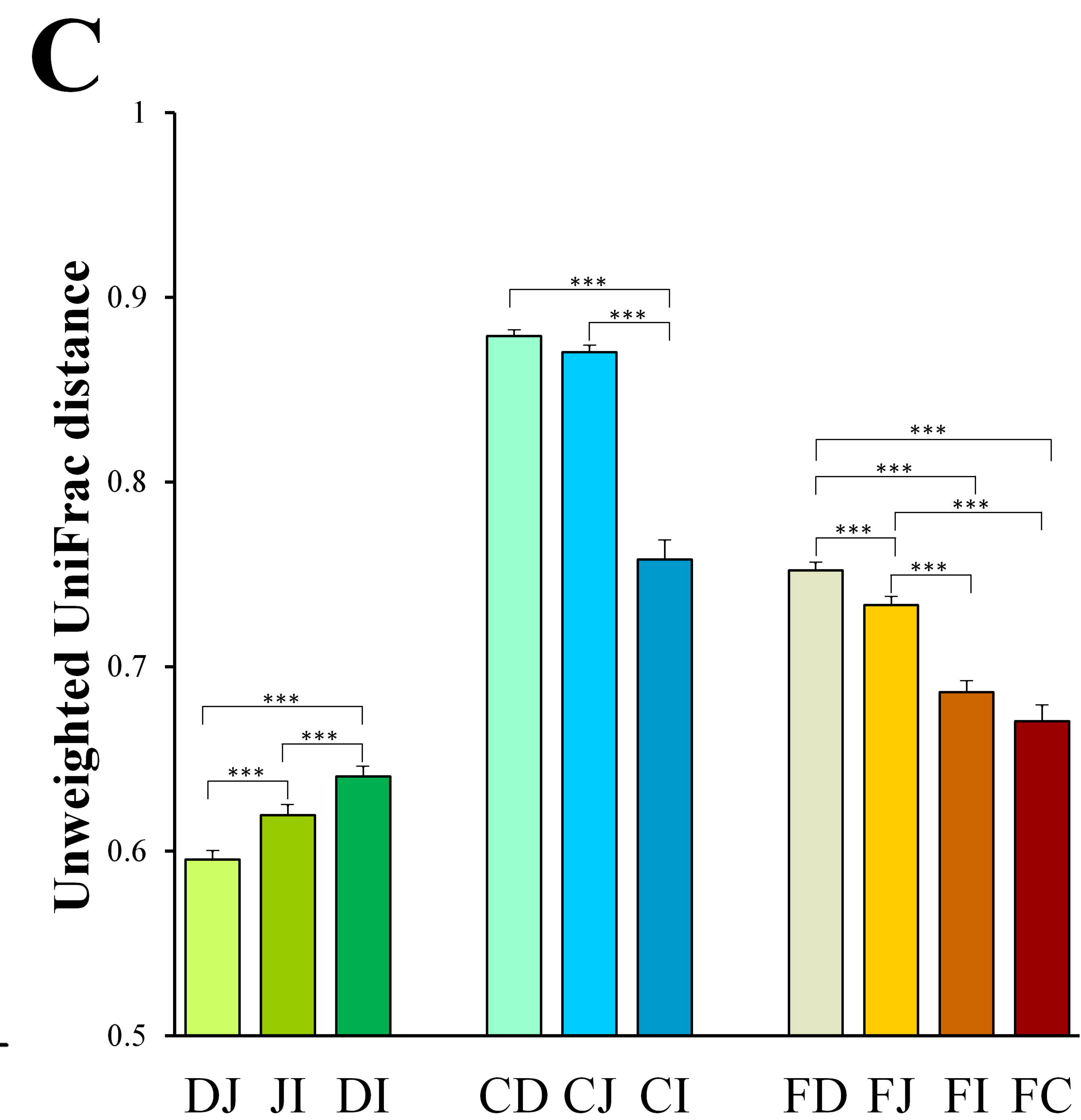

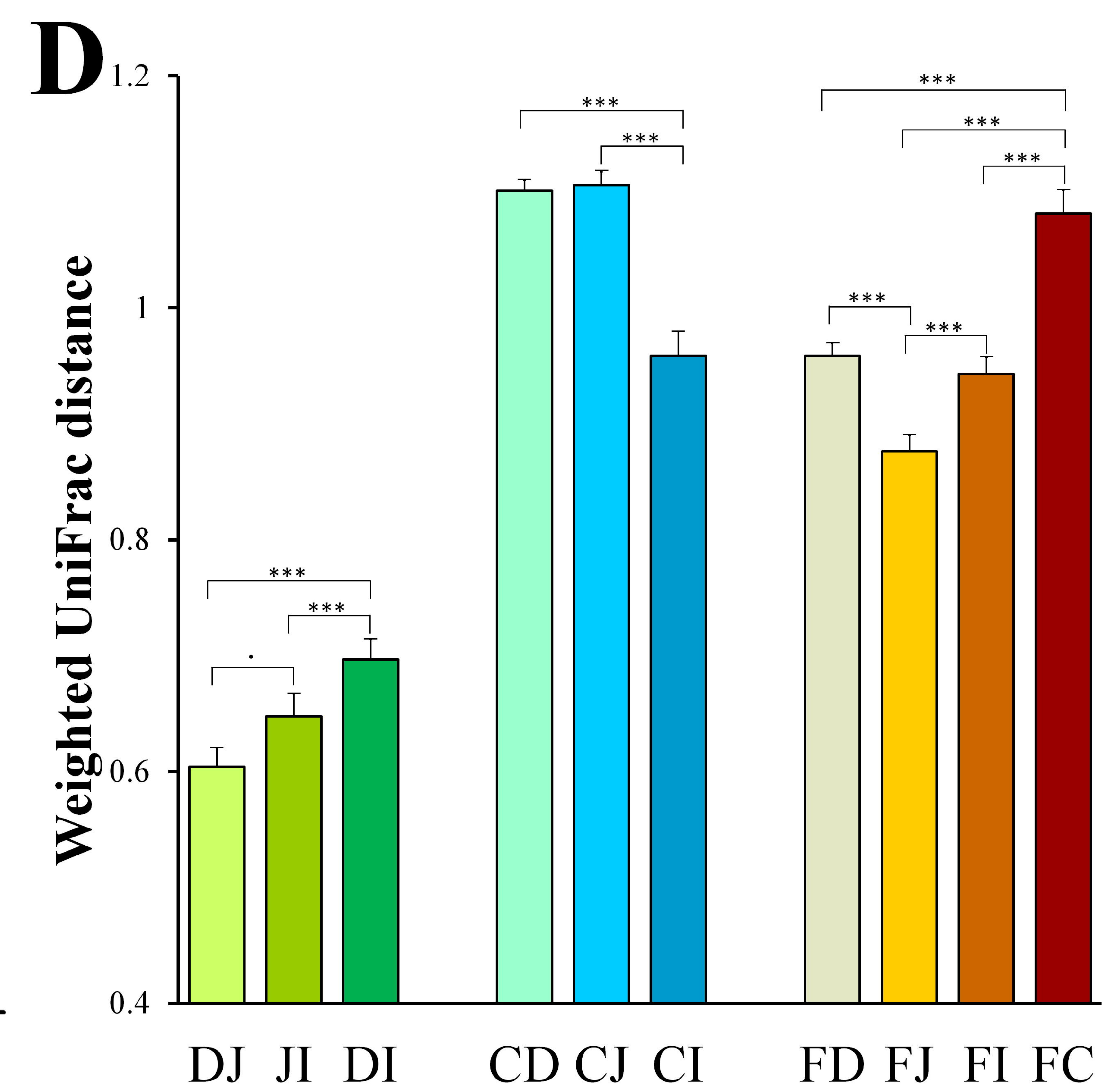

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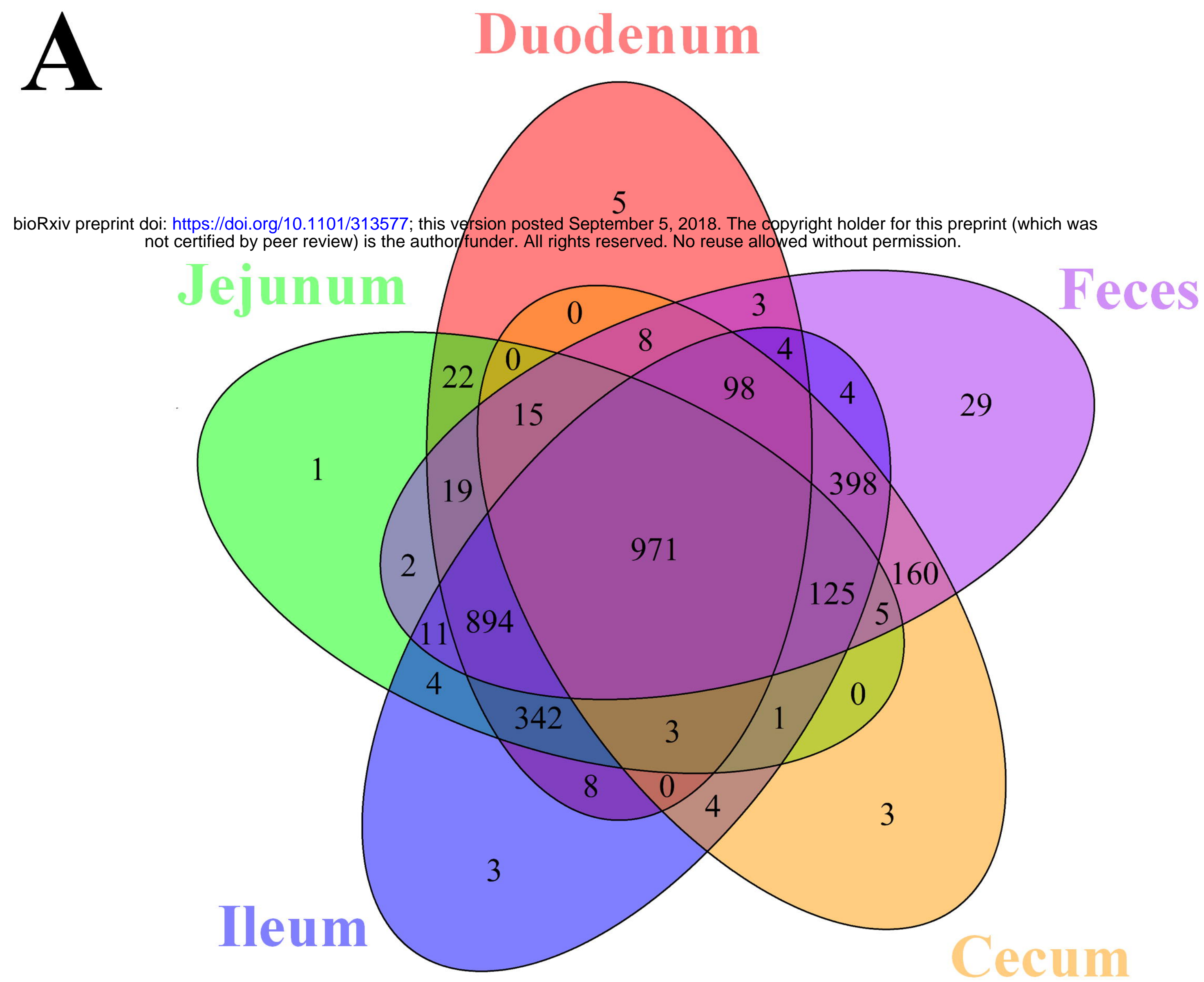

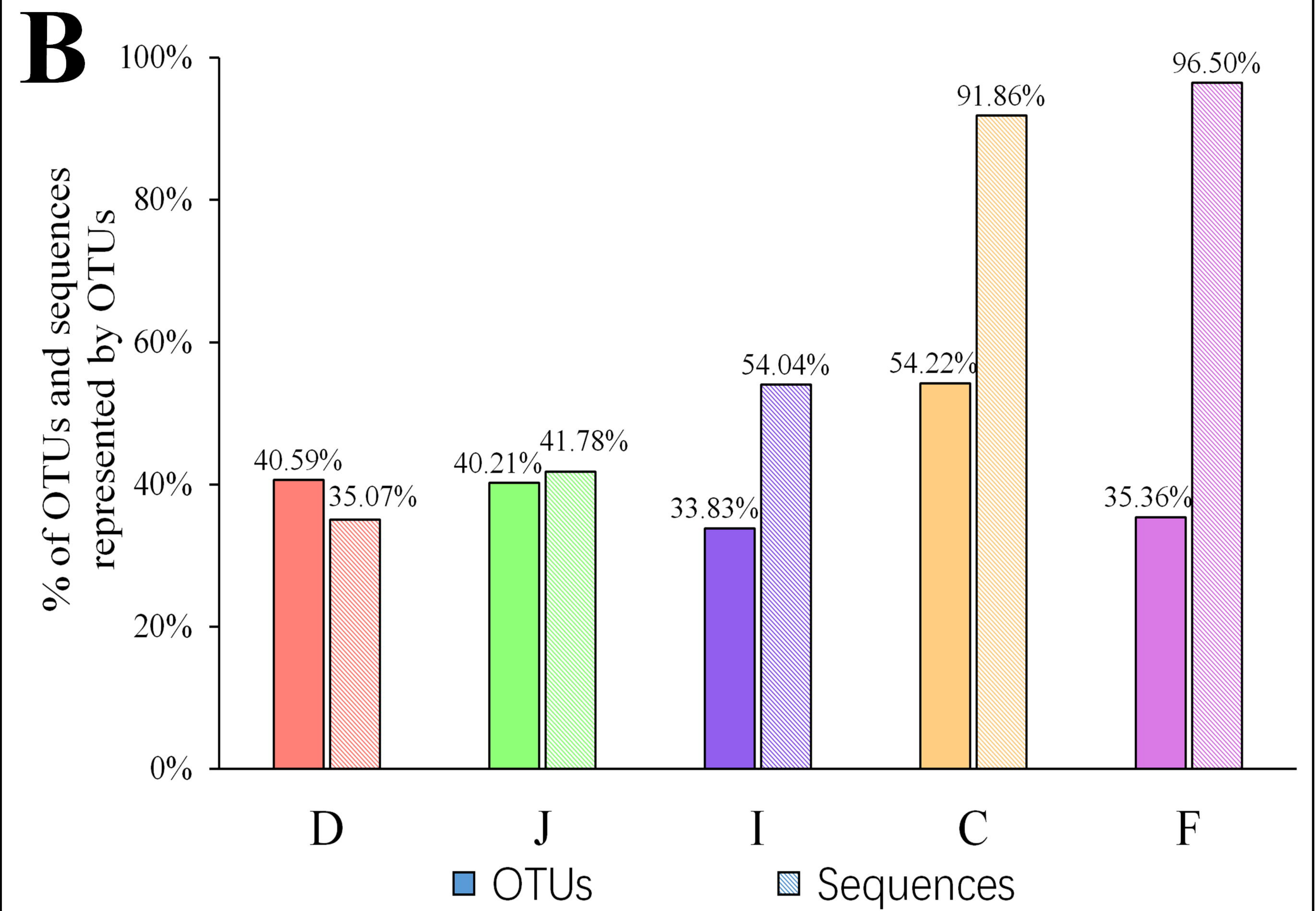

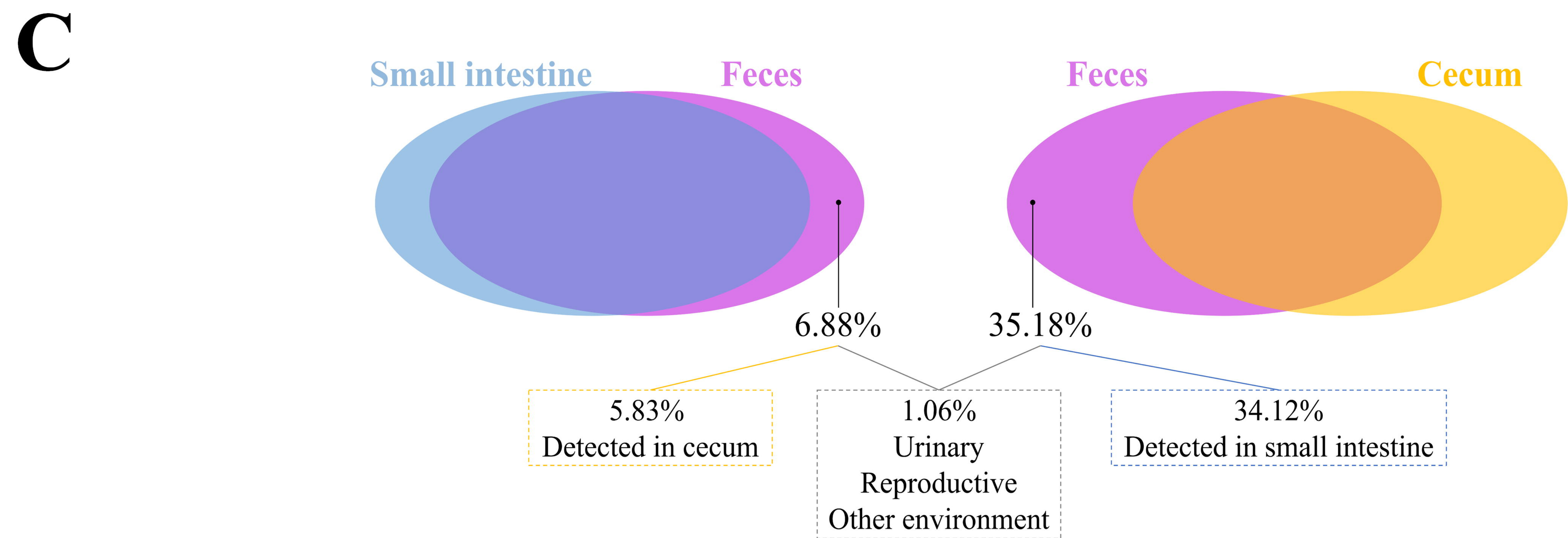

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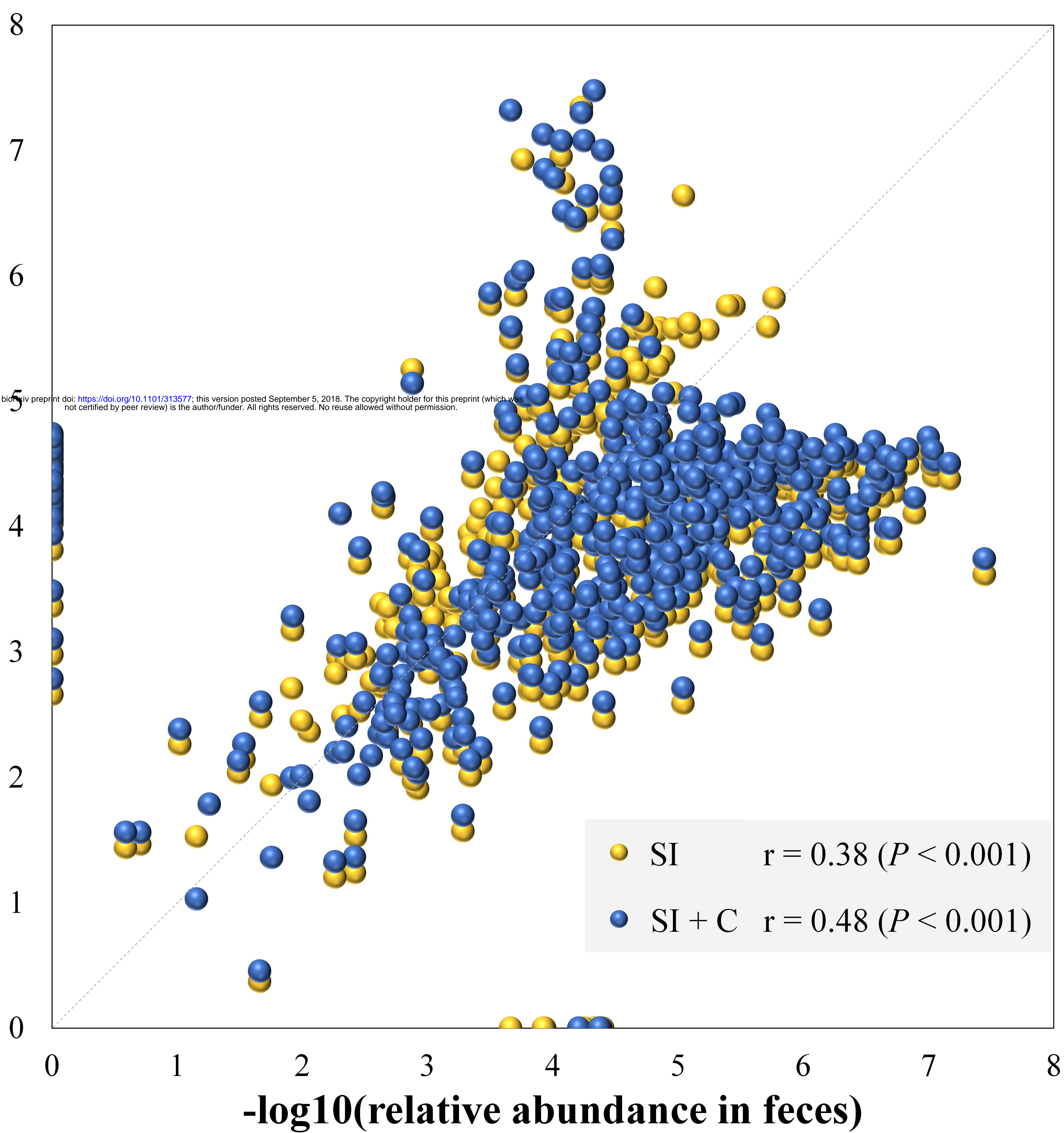

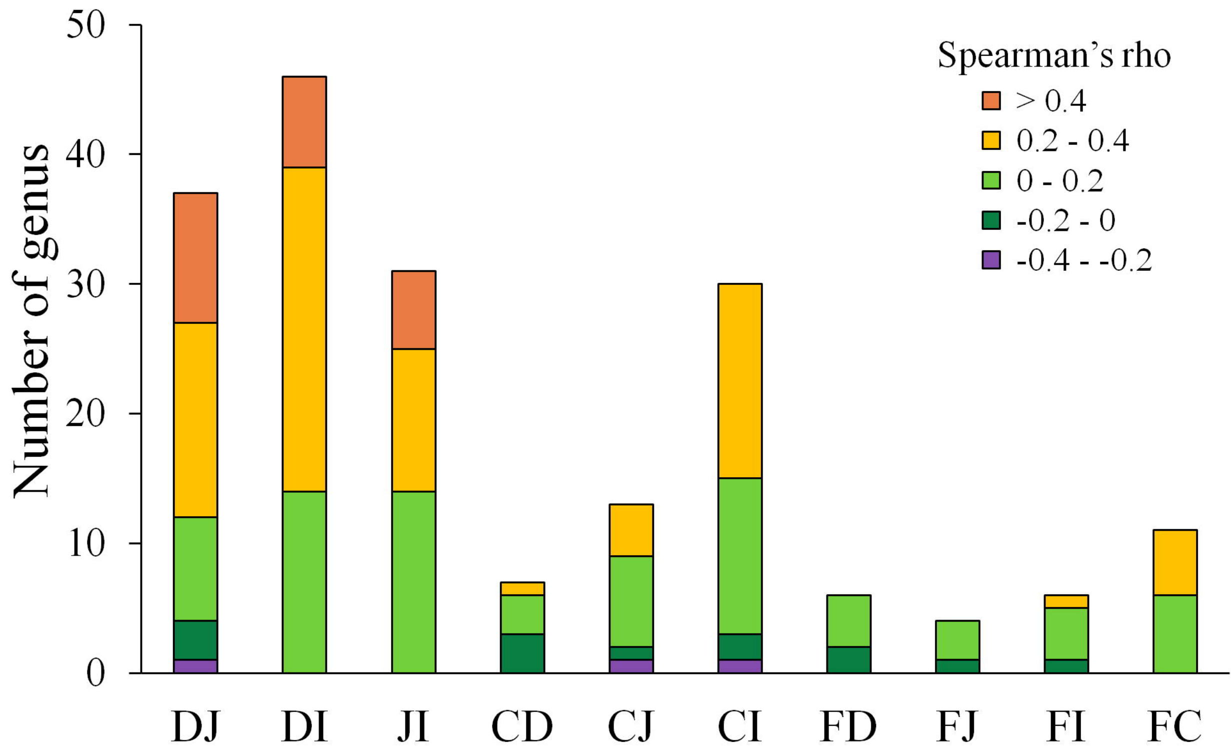
Number of genus
50
40
30
20
10
0
Spearman's rho
> 0.4
0.2 - 0.4
0 - 0.2
-0.2 - 0
-0.4 - -0.2
DJ
DI
JI
CD
CJ
CI
FD
FJ
FI
FC